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Short communication

A SIMPLE METHOD OF REDUCING THE FADING OF IMNUNOFLUORESCEN'CE DURING MICROSCOPY

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The phenomenon of fading of stained preparations on exposure to fluorescence-stimulating radiation has been an accepted feature of the immunofluorescent (IF) procedure since the definitive description of the technique by Coons and Kaplan (1950). Exposure to short-wave (ultra-violet) excitation as provided by high-pressure mercury vapour burners in common use results in very rapid fading, especially of fluorescein staining. The blue light emitted by quartz-halogen bulbs produces less rapid fading, but although these low-powered sources are entirely satisfactory (with appropriate microscope accessories) for viewing gross staining as seen for example, with tissue sections in screening tests for autoantibodies, more recent applications of IF involving the staining of specific determinants on cell surfaces and precise localisation of intracellular components and inclusions require maximum excitation by the more powerful light source.

We have found that the addition of p-phenylenediamine to the buffered glycerol used for mounting the stained preparations has a marked effect in retarding fading during microscopy. This is now routinely employed in this laboratory in the test for antibody to rheumatoid arthritis nuclear antigen (RANA) (Alspaugh and Tan, 1976) which involves the recognition of very fine speckled nuclear staining of EB virus-infected B lymphocytes (Raji or Wil_2 cells). It enables prolonged inspection of individual fields under a fluorescence microscope (Zeiss) equipped for incident illumination with an HBO 50 mercury burner, the installation having a direct short light-pathin order to produce maximum excitation. Only minimal fading of the fluorescent staining is discernible after 20 min continuous exposure, whereas marked bleaching occurs almost instantly with preparations mounted in conventional buffered glycerol. The improved mountant has also proved to be satisfactory in other systems involving cellular reactions and for tissue sections examined with mercury light excitation, and appears to have general applicability. An obvious further advantage is in the photographic recording of results.

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PREPARATION OF MOUNTANT

Add 10 ml of phosphate-buffered saline (0.01 M PO_4 pH 7.4 in 0.15 M NaCl) containing 100 mg of p-phenylenediamine (Hopkin and Williams, Romford, U.K.) to 90 ml of glycerol. The final pH should be adjusted to approximately 8.0 with 0.5 M carbonate-bicarbonate buffer pH 9.0.

Solutions containing p-phenylenediamine rapidly become brown on standing at room temperature and are then unsatisfactory for use in critical microscopy. This problem is overcome by storing the mixture in the dark at −20°C.

ACKNOWLEDGEMENT

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